ChIMP Vignette

Courtney Schiebout, H. Robert Frost

Load Libraries

Libraries "CAMML" (Schiebout and Frost 2022) and "Seurat" (Satija et al. 2015) need to be loaded to carry out this vignette. Packages will also load additional libraries they depend on.

```
library(CAMML)
library(Seurat)
library(dplyr)
```

Data Processing

The following code outlines how the joint scRNA-seq/CITE-seq data from Lawlor, et al. (2021) (Lawlor et al. 2021), available on the 10X Genomics website, was processed for further analysis.

```
#load data
malt <- Read10X("raw_feature_bc_matrix/")
```

```
## 10X data contains more than one type and is being returned as a list containing matrices of each typ
```

```
#isolate the RNA data and make it a Seurat Object
malt.data <- malt$`Gene Expression`
seurat <- CreateSeuratObject(counts = malt.data,  min.cells=10,min.features=100)

#filter for mitochondrial genes
seurat[["percent.mt"]] <- PercentageFeatureSet(seurat, pattern = "^MT-")
seurat <- subset(seurat, subset = percent.mt < 10)

#normalize and scale the RNA data
seurat <- NormalizeData(seurat)
seurat <- FindVariableFeatures(seurat, selection.method = "vst", nfeatures = 2000)
seurat <- ScaleData(seurat)
```

```
## Centering and scaling data matrix
```

```
#cluster and visualize
seurat <- RunPCA(seurat)
```

```
## PC_ 1
## Positive:  PCLAF, MKI67, RGS13, TYMS, MYBL2, CDK1, ZWINT, RRM2, UBE2C, AURKB
##     TK1, GRN, PKM, TOP2A, BIRC5, ACTB, CCNB2, PHGDH, DHFR, LMO2
##     NUF2, CST3, SPC25, CTSH, SERPINA9, ASPM, GTSE1, CDT1, SHCBP1, MAD2L1
## Negative:  ANXA1, GPR171, GZMK, CCL5, CD8A, SPRY1, GZMA, GTSCR1, RTKN2, TRGC2
##     NKG7, CD40LG, KRT1, KLRD1, IFNG, CRTAM, CD8B, LINC02446, ITM2A, LYPD3
##     ITGA6, ID1, CDKN1C, KLRB1, TRDC, TRGC1, ALKAL2, KLRC2, LINC01871, ENC1
## PC_ 2
## Positive:  PCLAF, MKI67, RRM2, ZWINT, CDK1, AURKB, UBE2C, TYMS, TOP2A, RGS13
##     BIRC5, TK1, SPC25, CDCA5, MYBL2, GTSE1, DHFR, NUF2, CDT1, CCNB2
##     CDCA7, MAD2L1, SERPINA9, RMI2, ASPM, CD79A, GINS2, CHEK1, ASF1B, SHCBP1
## Negative:  CEBPD, CST3, TNFAIP2, LYZ, TYROBP, CSF2RA, NDRG2, LGALS2, NECTIN2, FCER1G
##     SERPINA1, RAB32, G0S2, ETS2, PLAUR, ALDH2, IFITM3, C15orf48, CXCL8, VEGFA
##     AC020656.1, CLEC7A, IL1B, CXCL2, PKP2, DST, AIF1, TIMP1, PLXDC2, CFP
## PC_ 3
## Positive:  HLA-DRA, HLA-DQA1, HLA-DPA1, HLA-DQB1, HLA-DRB1, HLA-DPB1, CD74, IGHM, MS4A1, CD79A
##     HLA-DMA, IGKC, LY9, TCF4, BASP1, FCRL5, BCL2A1, TNFRSF13B, CTSZ, CD22
##     FTL, SWAP70, ID3, ITGAX, ARID3A, IGHA1, LDLRAD4, CTSH, SYNGR2, H3F3A
## Negative:  ITM2A, IL32, ANXA1, MAF, GZMK, CTLA4, BATF, LDHB, TIGIT, HMGB2
##     ICA1, MT2A, KLRB1, CORO1B, H2AFZ, CCL5, LDHA, NCOA7, GZMA, S100A4
##     TNFRSF4, NKG7, GPR171, MAGEH1, PCLAF, GAPDH, CH25H, S100A10, PTPN13, ID2
## PC_ 4
## Positive:  LYZ, TYROBP, LGALS2, FCER1G, CST3, AC020656.1, AIF1, IL1B, MS4A6A, CSF2RA
##     JAML, SERPINA1, CPVL, CFP, CD1E, AXL, G0S2, ITGAX, ID2, LST1
##     NLRP3, CLEC7A, EREG, C1QA, DUSP4, C15orf48, CLEC10A, CD4, PLAUR, C1QB
## Negative:  CD79A, MS4A1, IGHM, S100A16, DSP, TM4SF1, RBP1, HLA-DRA, FOXC1, S100A14
##     CDC42EP1, EDN1, SOX9, NFIB, KRT7, ELF3, CD74, PITX1, ADIRF, TRIM29
##     S100A2, KRT8, TJP1, CALD1, GABRP, PALMD, TACSTD2, RND3, MEIS2, MIA
## PC_ 5
## Positive:  PCLAF, MKI67, RRM2, BIRC5, CDK1, AURKB, LYZ, ZWINT, UBE2C, TCL1A
##     TOP2A, TK1, TYMS, LGALS2, SPC25, CDCA5, CST3, GTSE1, NUF2, CCNB2
##     AIF1, ASPM, IGLC2, ESCO2, AC020656.1, DHFR, C1orf162, MAD2L1, IL1B, PBK
## Negative:  DUSP4, ARID3A, TNFRSF13B, SLAMF7, CPEB4, IGHA1, TRPV3, NEAT1, PDGFA, CLNK
##     S100A4, LITAF, CD27, RAMP1, GUSB, RGS1, BCAR3, SPN, SQSTM1, UGCG
##     CYTOR, FCRL4, ACY3, CD63, YWHAH, VOPP1, RAB11FIP1, BSG, ACP5, CLECL1
```

```
seurat <- FindNeighbors(seurat, dims = 1:10)
```

```
## Computing nearest neighbor graph
```

```
## Computing SNN
```

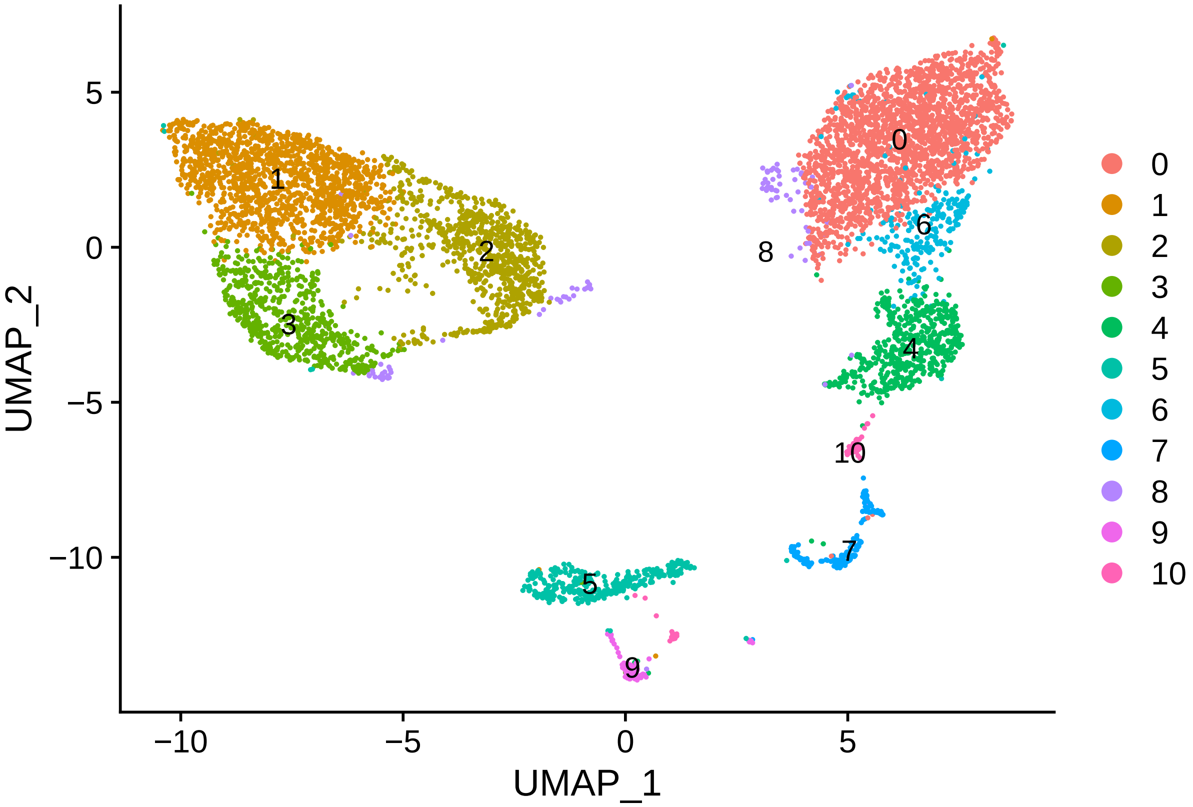
```
seurat <- FindClusters(seurat, resolution = 0.5)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 6438
## Number of edges: 216819
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8886
## Number of communities: 11
## Elapsed time: 0 seconds
```

```r
seurat <- RunUMAP(seurat, dims = 1:10)
```

```
## Warning: The default method for RunUMAP has changed from calling Python UMAP via reticulate to the R
## To use Python UMAP via reticulate, set umap.method to 'umap-learn' and metric to 'correlation'
## This message will be shown once per session

## 12:13:39 UMAP embedding parameters a = 0.9922 b = 1.112

## 12:13:39 Read 6438 rows and found 10 numeric columns

## 12:13:39 Using Annoy for neighbor search, n_neighbors = 30

## 12:13:39 Building Annoy index with metric = cosine, n_trees = 50

## 0%   10   20   30   40   50   60   70   80   90   100%

## [----|----|----|----|----|----|----|----|----|----|

## **************************************************|
## 12:13:40 Writing NN index file to temp file /var/folders/wv/9lqlnjl571q8w6tn77wg10pr0000gp/T//Rtmp0f
## 12:13:40 Searching Annoy index using 1 thread, search_k = 3000
## 12:13:41 Annoy recall = 100%
## 12:13:42 Commencing smooth kNN distance calibration using 1 thread
## 12:13:44 Initializing from normalized Laplacian + noise
## 12:13:44 Commencing optimization for 500 epochs, with 266746 positive edges
## 12:13:54 Optimization finished
```

```r
UMAPPlot(seurat, label = T)
```



scRNA-seq and CITE-seq Integration

Following the RNA data processing, the CITE-seq data needs to be added back into the data as an additional assay in the Seurat Object (Satija et al. 2015; Stoeckius et al. 2017). Since we filtered the data, the CITE-seq data needs aligned with the remaining cells.

```
#read in data and CITE-seq
cb <- malt$`Antibody Capture`

#filter CITE-seq
adt_assay <- CreateAssayObject(counts =
                                 cb[,colnames(cb) %in% colnames(seurat)])
```

```
## Warning: Feature names cannot have underscores ('_'), replacing with dashes
## ('-')
```

```
#add CITE-seq to SeuratObject
seurat[["ADT"]] <- adt_assay

#scale and normalize CITE-seq
seurat <- NormalizeData(seurat, assay = "ADT", normalization.method = "CLR")
```

```
## Normalizing across features
```

```
seurat <- ScaleData(seurat, assay = "ADT")
```

```
## Centering and scaling data matrix
```

Get Gene Sets and Run CAMML

In order to run CAMML and ChIMP, a gene set of cell types needs to be accessed. In the following code, "GetGeneSets" is used to load a pre-built gene set of 5 immune cell types. This can then be used to run CAMML. For this example HSCs will be removed.

```
#get gene sets
gene.set.df <- GetGeneSets(data = "immune.cells")

#filter out HSC
gene.set.df <- gene.set.df[-which(gene.set.df$cell.type == "HSC_CD34+"),]

gene.set.df
```

```
##    gene.symbol cell.type gene.weight      ensembl.id
## 1        ABCB4    B_cell    5.929227 ENSG00000005471
## 3         AIM2    B_cell    5.750254 ENSG00000163568
## 5        BANK1    B_cell    7.942142 ENSG00000153064
## 8          BLK    B_cell    7.548835 ENSG00000136573
## 9        BTNL9    B_cell    5.071020 ENSG00000165810
## 12        CD19    B_cell    8.853964 ENSG00000177455
## 13        CD22    B_cell    6.364610 ENSG00000012124
## 19       CD79A    B_cell    8.582138 ENSG00000105369
## 24       CPNE5    B_cell    6.580999 ENSG00000124772
## 30        E2F5    B_cell    7.159631 ENSG00000133740
## 32       FCRL1    B_cell    8.492002 ENSG00000163534
## 33       FCRL2    B_cell    6.669676 ENSG00000132704
## 34       FCRLA    B_cell    8.898029 ENSG00000132185
## 38     HLA-DOB    B_cell    7.261022 ENSG00000241106
## 55   LINC00926    B_cell    5.223783 ENSG00000247982
## 60       MS4A1    B_cell    7.927719 ENSG00000156738
## 66       P2RX5    B_cell    5.862599 ENSG00000083454
## 69     PKHD1L1    B_cell    6.031826 ENSG00000205038
## 70     PLEKHG1    B_cell    5.831675 ENSG00000120278
## 71        PNOC    B_cell    7.489833 ENSG00000168081
## 74     RALGPS2    B_cell    5.228555 ENSG00000116191
## 85        SPIB    B_cell    6.160680 ENSG00000269404
## 86       STAP1    B_cell    5.787053 ENSG00000035720
## 92       TLR10    B_cell    7.109604 ENSG00000174123
## 2       ADGRE2  Monocyte    7.729049 ENSG00000127507
## 11       C5AR1  Monocyte    8.813094 ENSG00000197405
## 14     CD300LF  Monocyte    6.470491 ENSG00000186074
## 18        CD68  Monocyte    5.983464 ENSG00000129226
## 22      CDKN1C  Monocyte    5.755567 ENSG00000129757
## 25      CPPED1  Monocyte    5.344299 ENSG00000103381
## 27       CSF1R  Monocyte    6.984429 ENSG00000182578
## 28      CXCL16  Monocyte    6.330266 ENSG00000161921
## 36         HCK  Monocyte    7.334923 ENSG00000101336
## 37         HK3  Monocyte    5.814265 ENSG00000160883
## 51      LILRA1  Monocyte    6.479104 ENSG00000104974
## 52      LILRA2  Monocyte    6.321271 ENSG00000239998
## 53      LILRA5  Monocyte    5.485005 ENSG00000187116
## 54      LILRB2  Monocyte    8.092027 ENSG00000131042
## 57        LST1  Monocyte    5.607879 ENSG00000204482
## 61       MS4A7  Monocyte    6.592873 ENSG00000166927
## 62        MSR1  Monocyte    6.644375 ENSG00000038945
## 67      PAPSS2  Monocyte    5.102467 ENSG00000198682
## 68       PILRA  Monocyte    7.658938 ENSG00000085514
## 77    SERPINA1  Monocyte    6.364474 ENSG00000197249
## 80     SLC31A2  Monocyte    5.431567 ENSG00000136867
## 81      SLC7A7  Monocyte    5.888768 ENSG00000155465
## 83     SMPDL3A  Monocyte    5.007270 ENSG00000172594
## 84        SPI1  Monocyte    5.337993 ENSG00000066336
## 87      TBC1D8  Monocyte    5.634734 ENSG00000204634
## 88      TBXAS1  Monocyte    5.145335 ENSG00000059377
## 97        VMO1  Monocyte    7.072633 ENSG00000182853
## 23       CLIC3   NK_cell    7.119036 ENSG00000169583
## 31       FASLG   NK_cell    5.610501 ENSG00000117560
## 40     IL18RAP   NK_cell    5.080865 ENSG00000115607
## 43     KIR2DL4   NK_cell    5.716879 ENSG00000189013
## 44     KIR3DL1   NK_cell    6.315612 ENSG00000167633
## 45     KIR3DL2   NK_cell    5.439694 ENSG00000240403
## 46       KLRF1   NK_cell    5.481712 ENSG00000150045
## 48       KRT86   NK_cell    5.458481 ENSG00000170442
## 73       PRR5L   NK_cell    5.241528 ENSG00000135362
## 76       S1PR5   NK_cell    5.062385 ENSG00000180739
## 78      SH2D1B   NK_cell    7.356306 ENSG00000198574
## 82      SLFN13   NK_cell    5.210182 ENSG00000154760
## 98        XCL1   NK_cell    6.650246 ENSG00000143184
## 99        YES1   NK_cell    5.168530 ENSG00000176105
## 6       BCL11B   T_cells    5.564075 ENSG00000127152
## 15        CD3D   T_cells    6.559457 ENSG00000167286
## 16        CD3E   T_cells    5.457156 ENSG00000198851
## 17        CD3G   T_cells    7.904570 ENSG00000160654
## 20        CD8A   T_cells    5.715826 ENSG00000153563
## 21        CD8B   T_cells    7.828488 ENSG00000172116
## 29        DPP4   T_cells    5.142898 ENSG00000197635
## 39        ICOS   T_cells    6.000820 ENSG00000163600
## 41      INPP4B   T_cells    5.664718 ENSG00000109452
## 42         ITK   T_cells    5.013501 ENSG00000113263
## 47       KLRG1   T_cells    5.244996 ENSG00000139187
## 50        LEF1   T_cells    5.500641 ENSG00000138795
## 56       LRRN3   T_cells    7.577637 ENSG00000173114
## 58         MAL   T_cells    7.694320 ENSG00000172005
## 63       NELL2   T_cells    6.411428 ENSG00000184613
## 65      OXNAD1   T_cells    5.250065 ENSG00000154814
## 75      RNF157   T_cells    5.376098 ENSG00000141576
## 79       SIRPG   T_cells    5.538280 ENSG00000089012
## 89        TC2N   T_cells    5.002518 ENSG00000165929
## 90        TCF7   T_cells    5.961207 ENSG00000081059
## 91      THEMIS   T_cells    6.889632 ENSG00000172673
## 93     TRABD2A   T_cells    5.447930 ENSG00000186854
## 94        TRAC   T_cells    5.025658 ENSG00000277734
## 95       TRAT1   T_cells    7.189648 ENSG00000163519
## 96     UBASH3A   T_cells    5.305995 ENSG00000160185
```

```
#run CAMML
seurat <- CAMML(seurat, gene.set.df)
```

```
## Computing VAM distances for 4 gene sets, 6438 cells and 15518 genes.
```

```
## Min set size: 11, median size: 23.5
```

```
## Warning: Feature names cannot have underscores ('_'), replacing with dashes
## ('-')
```

```
## Warning: Feature names cannot have underscores ('_'), replacing with dashes
## ('-')
```

```
## Warning: Cannot add objects with duplicate keys (offending key: vamcdf_) setting
## key to original value 'camml_'
```

Integrate CITE-seq via ChIMP into CAMML with k-means Discretization

Following the running of CAMML, the cell type scores can be altered by the inclusion of CITE-seq data using ChIMP. In order to use this, a list of cell types and their corresponding CITE-seq markers needs to be built. This list, the Seurat Object, and a vector of booleans will then be fed into ChIMP. The vector serves to designate whether, in cases of multiple marker proteins in a cell type, any marker protein can be present to maintain the CAMML score or if ChIMP should require all marker proteins to be present to maintin the CAMML score.

In other words, if a user designates both CD4 and CD8 for T cells, anyMP=TRUE would require that just one of the two markers be present in a cell for the cell to have a nonzero cell type score. However, if anyMP=FALSE, both markers would have to be present in a cell for the cell type score to be nonzero.

In this example, we use anyMP=FALSE for monocytes to single out cells that are positive for both CD14 and CD16. We use anyMP=TRUE to select for T cells that are either CD4 or CD8 positive.

```
#compare ADT markers and cell types
rownames(seurat@assays$ADT)
```

```
##  [1] "CD3-TotalSeqB"           "CD4-TotalSeqB"
##  [3] "CD8a-TotalSeqB"          "CD14-TotalSeqB"
##  [5] "CD15-TotalSeqB"          "CD16-TotalSeqB"
##  [7] "CD56-TotalSeqB"          "CD19-TotalSeqB"
##  [9] "CD25-TotalSeqB"          "CD45RA-TotalSeqB"
## [11] "CD45RO-TotalSeqB"        "PD-1-TotalSeqB"
## [13] "TIGIT-TotalSeqB"         "CD127-TotalSeqB"
## [15] "IgG2a-control-TotalSeqB" "IgG1-control-TotalSeqB"
## [17] "IgG2b-control-TotalSeqB"
```

```r
rownames(seurat@assays$CAMML)
```

```
## [1] "B-cell"   "Monocyte" "NK-cell"  "T-cells"
```

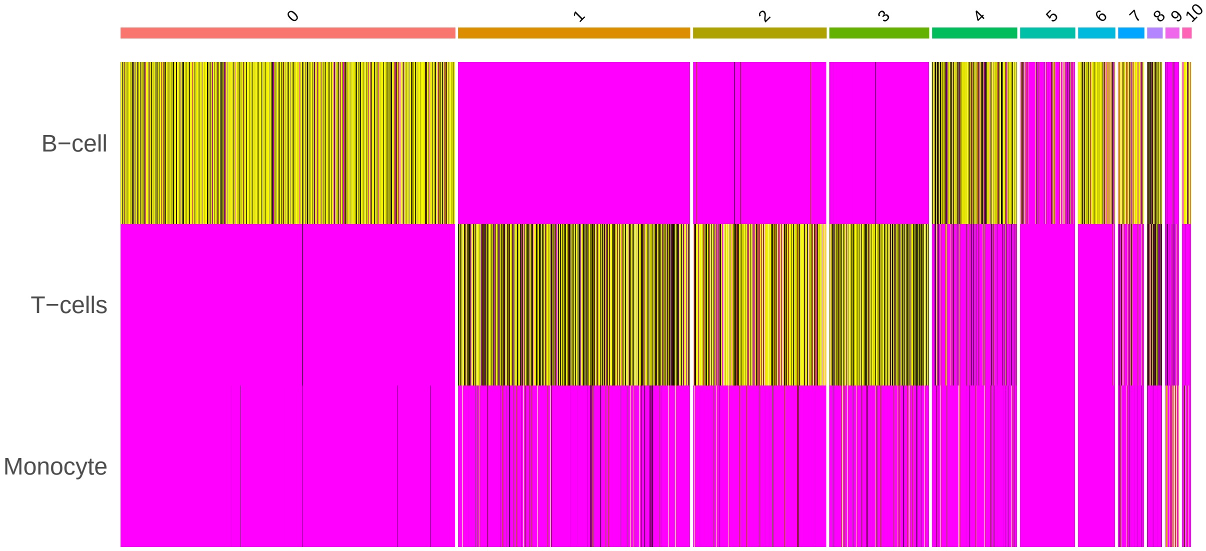
```r
#create CITE list
markers <- cbind(c(rownames(seurat),rownames(seurat)[2],rownames(seurat)[4]),
      (rownames(seurat@assays$ADT)[c(8,4,7,2,6,3)]))
citelist <- list()
for (i in 1:length(rownames(seurat))){
  citelist[[i]] <- markers[which(markers[,1]==rownames(seurat)[i]),2]
}
names(citelist) <- rownames(seurat)
citelist
```

```
## $`B-cell`
## [1] "CD19-TotalSeqB"
##
## $Monocyte
## [1] "CD14-TotalSeqB" "CD16-TotalSeqB"
##
## $`NK-cell`
## [1] "CD56-TotalSeqB"
##
## $`T-cells`
## [1] "CD4-TotalSeqB"  "CD8a-TotalSeqB"
```

```r
#run ChIMP
seuratk <- ChIMP(seurat, citelist, anyMP = c(T,F,T,T))

#visualize the cell type scores
seurat.markers = FindAllMarkers(seuratk, assay="ChIMP", only.pos = TRUE)
```

```
## Calculating cluster 0

## Calculating cluster 1

## Calculating cluster 2

## Calculating cluster 3

## Calculating cluster 4

## Calculating cluster 5

## Warning in FindMarkers.default(object = data.use, slot = data.slot, counts =
## counts, : No features pass logfc.threshold threshold; returning empty data.frame

## Calculating cluster 6

## Calculating cluster 7
## Calculating cluster 8

## Warning in FindMarkers.default(object = data.use, slot = data.slot, counts =
## counts, : No features pass logfc.threshold threshold; returning empty data.frame

## Calculating cluster 9

## Calculating cluster 10

## Warning in FindMarkers.default(object = data.use, slot = data.slot, counts =
## counts, : No features pass logfc.threshold threshold; returning empty data.frame
```

```
DefaultAssay(object = seuratk) = "ChIMP"
top.pathways <- seurat.markers %>% group_by(cluster) %>% top_n(n = 3, wt = avg_log2FC)
DoHeatmap(seuratk, slot="data", features = top.pathways$gene,
          size=2, label=T, raster = F) + NoLegend()
```



Integrate CITE-seq via ChIMP into CAMML with Quantile Discretization

This example follows the same pipeline as the above example but uses a median discretization for CITE-seq instead of k-means for comparison.

```
#compare ADT markers and cell types
rownames(seurat@assays$ADT)
```

```
##  [1] "CD3-TotalSeqB"           "CD4-TotalSeqB"
##  [3] "CD8a-TotalSeqB"          "CD14-TotalSeqB"
##  [5] "CD15-TotalSeqB"          "CD16-TotalSeqB"
##  [7] "CD56-TotalSeqB"          "CD19-TotalSeqB"
##  [9] "CD25-TotalSeqB"          "CD45RA-TotalSeqB"
## [11] "CD45RO-TotalSeqB"        "PD-1-TotalSeqB"
## [13] "TIGIT-TotalSeqB"         "CD127-TotalSeqB"
## [15] "IgG2a-control-TotalSeqB" "IgG1-control-TotalSeqB"
## [17] "IgG2b-control-TotalSeqB"
```

```
rownames(seurat@assays$CAMML)
```

```
## [1] "B-cell"   "Monocyte" "NK-cell"  "T-cells"
```

```
#create CITE list
markers <- cbind(c(rownames(seurat),rownames(seurat)[2],rownames(seurat)[4]),
      (rownames(seurat@assays$ADT)[c(8,4,7,2,6,3)]))
citelist <- list()
for (i in 1:length(rownames(seurat))){
  citelist[[i]] <- markers[which(markers[,1]==rownames(seurat)[i]),2]
}
names(citelist) <- rownames(seurat)
citelist
```

```
## $`B-cell`
## [1] "CD19-TotalSeqB"
##
## $Monocyte
## [1] "CD14-TotalSeqB" "CD16-TotalSeqB"
##
## $`NK-cell`
## [1] "CD56-TotalSeqB"
##
## $`T-cells`
## [1] "CD4-TotalSeqB"  "CD8a-TotalSeqB"
```

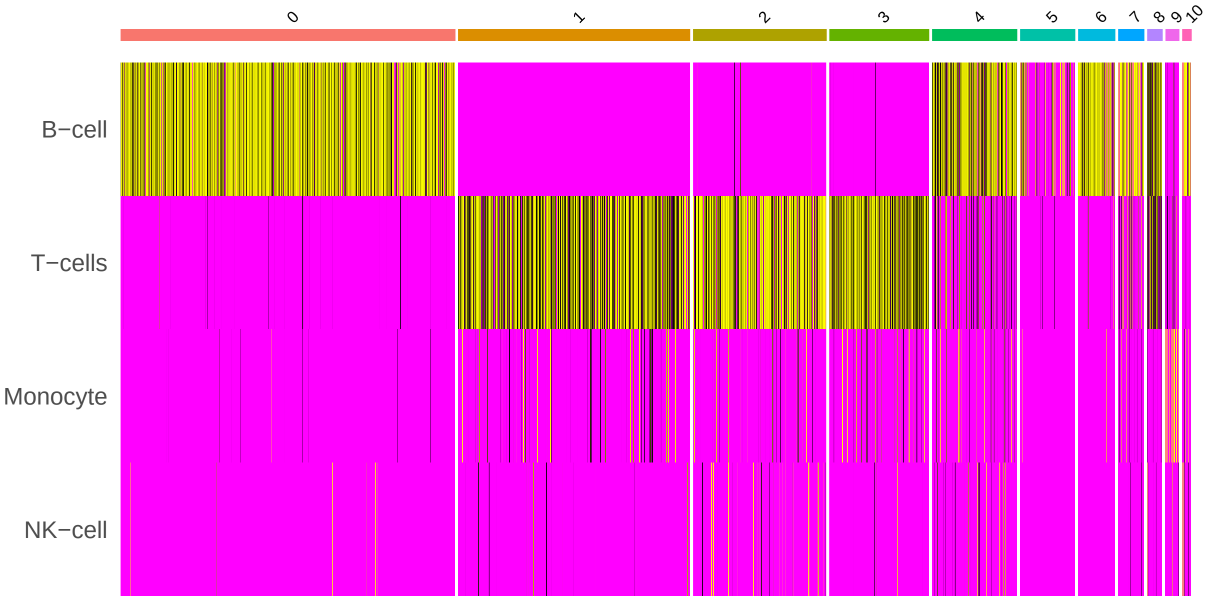
```
#run ChIMP
seuratq <- ChIMP(seurat, citelist, method = "q", anyMP = c(T,F,T,T))

#visualize the cell type scores
seurat.markers = FindAllMarkers(seuratq, assay="ChIMP", only.pos = TRUE)
```

```
## Calculating cluster 0
```

```
## Calculating cluster 1
```

```
## Calculating cluster 2
## Calculating cluster 3

## Calculating cluster 4

## Calculating cluster 5

## Warning in FindMarkers.default(object = data.use, slot = data.slot, counts =
## counts, : No features pass logfc.threshold threshold; returning empty data.frame

## Calculating cluster 6

## Calculating cluster 7

## Calculating cluster 8

## Warning in FindMarkers.default(object = data.use, slot = data.slot, counts =
## counts, : No features pass logfc.threshold threshold; returning empty data.frame

## Calculating cluster 9

## Calculating cluster 10
```

```
DefaultAssay(object = seuratq) = "ChIMP"
top.pathways <- seurat.markers %>% group_by(cluster) %>% top_n(n = 3, wt = avg_log2FC)
DoHeatmap(seuratq, slot="data", features = top.pathways$gene,
          size=2, label=T, raster = F) + NoLegend()
```



References

Lawlor, Nathan, Djamel Nehar-Belaid, Jessica D.S. Grassmann, Marlon Stoeckius, Peter Smibert, Michael L. Stitzel, Virginia Pascual, Jacques Banchereau, Adam Williams, and Duygu Ucar. 2021. "Single Cell Analysis of Blood Mononuclear Cells Stimulated Through Either LPS or Anti-CD3 and Anti-CD28." *Frontiers in Immunology* 12 (March): 636720. https://doi.org/10.3389/fimmu.2021.636720.

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